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ARTICLE

Analysis of *Salmonella* Contaminated Beef Odor Using an Electronic Nose

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Abstract

An electronic nose was used to identify *Salmonella* contamination on beef based on odors. To detect pathogen contamination of beef, 100 μL of 10^5 CFU/g *Salmonella* Enteritidis or *Salmonella* Typhimurium cell suspensions were spiked onto 5 g beef sirloin samples in individual vials. Odor changes over time were then measured and analyzed using an electronic nose system to identify pathogen contamination. In principle, the electronic nose system based on a surface acoustic wave (SAW) detector produced different frequency responses depending on the time and amount of each chemical. Multivariate analysis of the odor data was conducted to detect *Salmonella* contamination of beef. *Salmonella* odors were successfully distinguished from uncontaminated beef odors by principal component analysis (PCA). The PCA results showed that *Salmonella* contamination of beef could be detected after 4 h of incubation. The numbers of cells enumerated by standard plate count after 4 h of inoculation were 2×10^6 CFU/g for both *Salmonella* Enteritidis and *Salmonella* Typhimurium.

Key words: Electronic nose, *Salmonella*, Odor analysis, PCA

Introduction

Increasing public awareness for food safety makes quality evaluation of meat, poultry, and their products more important. Since olfaction is important in evaluation of the quality and safety of foods, many efforts have been made to develop an instrument that mimics the olfactory system in the nose. The instrument that operates on the similar principle as human nose is called an electronic nose.

Electronic noses are based on the array of artificial receptors. Although they differ from each other in the operating principle, the number of sensors, and the sensitivity and selectivity, their function is same. They detect volatile or semi-volatile vapors by means of sensors and send signals to recognition units to interpret the signal pattern. A typical electronic nose system consists of a chemical sensor array, electronics, pumps, an air conditioner, a flow controller, and software for hardware controlling and data processing.

Electronic nose systems have several advantages over conventional analyses of volatile compounds by traditional analytical techniques and by human sensory panel methods. The conventional analytical techniques such as gas chromatography/mass spectrometry (GC/MS) allow high accuracy and low detection limits, but are expensive and time and labor consuming. While sensory methods are subjective and have poor reproducibility. Even though electronic noses are not complete replacement of the sensory methods or the analytical techniques, they combine advantages of the two disciplines. These electronic nose systems are objective and produce reproducible analysis resulting in a relatively short time.

Electronic noses have been used in the quality and freshness evaluation of foods which is related to the food safety. O'Connell *et al*. (2001), who used a simple electronic nose to determine the fish freshness of Argentinean hake. They could identify between fresh and old samples in terms of storage days in a refrigerator. Storey *et al*. (2001) applied solid state gas sensors to the measurement of the trimethylamine and dimethylamine generated from spoilage of fish and fish products. Grigioni *et al*. (2000) identified warmed-over flavour (WOF) aroma in low temperature-long time processed meat. The WOF odor could be classified by using an electronic nose based on conduction polymer sensors. Natale *et al*. (2001) combined two electronic nose

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systems to increase the evaluation performance of codfish freshness. Barbri *et al*. (2008) successfully assessed freshness of sardines stored at 4°C with an electronic nose composed of six tin oxide gas sensors and a multi-class classifier model. Detection of bacteria spoilage of foods using an electronic nose was also investigated (Arnold and Senter, 1998; Blixt and Borch, 1999; Keshri *et al*., 1998; McEntegart *et al*., 2000). By using an electronic nose based on metal oxide semiconductor (MOS) sensors, Rossi *et al*. (1995) could correctly classified 90.5% of odors generated by bacteria grown on agar medium. The researchers also detected and classified pathogenic strains sometimes found in meat product by using the electronic nose (Rossi *et al*., 1996). Dutta *et al*. (2002) used an electronic nose comprising an array of thirty-two gas sensors to identify six species of bacteria responsible for eye infections. They could classify the bacteria with 96% and 98% of accuracy with Self Organizing Map and Radial basis function network, respectively.

The goal of this study was to investigate the feasibility of using an electronic nose for the detection of *Salmonella* contamination in beef. Contamination of pathogenic bacteria onto beef was measured by an electronic nose and analyzed by multivariate data analysis technique. Principal component analysis (PCA) was used to reduce the dimension of the odor chromatogram and to determine odor of *Salmonella* Enteritidis or *Salmonella* Typhimurium contaminated beef. Also, qualitative analysis of the volatile organic compound isolated from the headspace of *Salmonella* Enteritidis or *Salmonella* Typhimurium was performed with a mass spectrometer.

Materials and Methods

Beef sample preparation

Beef sirloins were obtained from a local market. The sirloins were cut into 20 mm thick steak and packed in sanitized vinyl bags. The sample bags were stored in a refrigerator at 5°C before experiments. Only the muscle part of the meats was used for odor measurements to acquire uniform odors.

Bacteria and enrichment media

Salmonella Enteritidis ATCC 14028 and *Salmonella* Typhimurium ATCC 13076 were acquired from American Type Culture Collection (ATCC). All fresh cultures for experiments were obtained by inoculating freeze-dried cultures into enrichment broth and incubating them at 37°C for 24 h. For both *Salmonella* Enteritidis and *Salmonella* Typhimurium (*S*. Typhimurium) cell enrichment, brain heart infusion (BHI) broth (Difco Laboratories, USA) was used.

Electronic nose system

Electronic nose system used in this study was zNose 7100 (Electronic Sensor Technology, USA). The zNose is based on gas chromatography (GC) and a surface acoustic wave (SAW) detector. Unlike conventional GC, the one in the zNose is a portable GC, which uses a direct heated 1 m capillary column to make the system smaller and to achieve fast analysis.

In principle, odors or vapors from samples enter the system through a temperature controlled inlet and are preconcentrated for a specific time in a Tenax trap. The preconcentrated vapors are swept by helium carrier gas into the temperature programmable column. The column temperature is programmed to follow a linear rise to its maximum temperature. The linear temperature rise causes the different chemical components to be released and travel through the column with a different relative velocity.

The SAW detector consists of an uncoated acoustic resonator bonded to a Peltier thermoelectric heat pump to heat or to cool the quartz substrate. The vapors dispersed from the column are passed to the SAW detector, which produces frequency response depends on the time and amount of each chemical. Control software of the electronic nose system displays the sensor frequency and derivative-of-frequency which is equivalent to a conventional chromatogram.

For testing the beef samples, the column was ramped from 30 to 150°C at 5°C/sec and the detector was held at 30°C during the analysis. The sampling time was 10 sec and the same conditions were maintained during the experiments.

Odor analysis procedure

To measure the headspace chemistry of beef samples for detection of *Salmonella* contamination, each of 5 g aliquots was cut from the stored beef specimen and placed into a 40 mL septa-sealed vial. The beef aliquot was inoculated using 100 μL of 10^5 CFU/g *Salmonella* Enteritidis or *Salmonella* Typhimurium cell suspensions. And then, the vial was capped and placed into a dry bath at 37°C until odor measurements. Vapor samples were collected and preconcentrated by inserting a side-ported sampling needle through the septa at every 1 h. The preconcentrated vapors were then injected and analyzed by the electronic nose as shown in Fig. 1. The entire mea-

Fig. 1. The SAW based an electronic nose system for detecting *Salmonella* odor from beef sample.

suring process took approximately 1 min per sample.

Odor data analysis

Odor analyses were performed using multivariate analysis techniques. A PCA technique was used to reduce the dimension of the odor chromatogram. Odors were classified by PCA to differentiate odor changes resulting from *Salmonella* growth. For the multivariate analysis, Unscrambler 9.7 (CAMO, Norway) was used.

Analysis of volatile metabolites

The headspace volatile compounds produced by Salmonella growth were collected using solid-phase microextraction (SPME) fibers (50/30 μm DVB/CAR/PDMS, 65 μm PDMS/DVB) and analyzed by gas chromatography-mass spectrometry (GC/MS, QP 5050A, Shidmazu, Japan) operated in the electron-ionisation (EI) mode. Headspace vapors were collected from BHI broth instead of beef to reduce the odor variation caused by beef itself. *Salmonella* Enteritidis or *Salmonella* Typhimurium was incubated in brain heart infusion (BHI) broth at 37°C for 24 h. And then, the absorption of the compounds to the SPME fiber was performed at 37°C for 30 min. The desorption time from SPME fiber for GC/MS measurement was 3 min with an inlet temperature of 250°C. The GC column was an 30 m×0.25 mm (id), 0.25 μm film thickness ValcoBond VB-5 (Valco Instruments, Switzerland). Helium was used as the carrier gas with flow rate of 1.0 mL/min. The GC method used following conditions: initial oven temperature of 40°C for 5 min, increasing to 250°C at 15°C/min, and then held at 250°C for 10 min; detector temperature 270°C.

Results and Discussion

Detection of *Salmonella* contaminated beef was tested by analyzing the responses of the electronic nose to different growth stages of *S.* Enteritidis or *S.* Typhimurium. *S.* Enteritidis or *S.* Typhimurium cells were spiked onto the beef and the odors were measured with the electronic nose. Chromatographic analysis of odors showed distinctive patterns for each bacterium. Fig. 2 and Fig. 3 show the response curves of the electronic nose to different growth stages of *S.* Enteritidis or *S.* Typhimurium, respectively.

At an early growth stage of 1 h, there was no noticeable peak and no distinctive odor difference between responses of different *Salmonella* strains. As growth time increases, peaks were emerged at a certain retention time (RT). The highest peak appeared to be at RTs of 23.8-24.8 for the *Salmonella* contaminated beef. Peaks were emerged at 2 h after incubation. However, intensities of response peaks

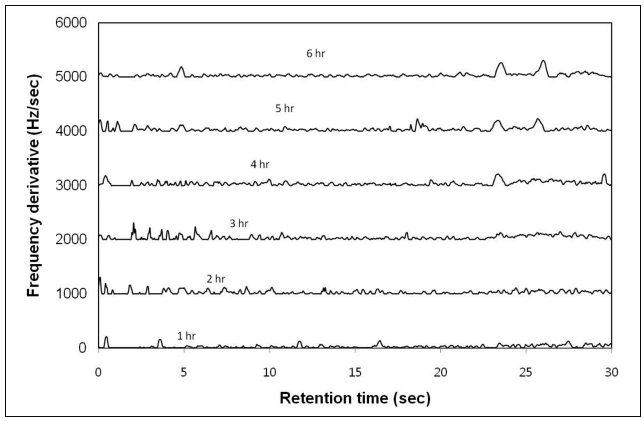

Fig. 2. Response curves of the electronic nose on growth of *S.* Enteritidis spiked onto beef.

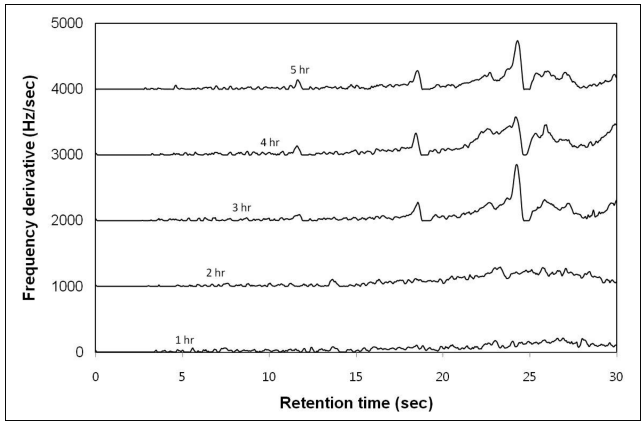

Fig. 3. Response curves of the electronic nose on growth of *S.* Typhimurium spiked onto beef (Curves marked 2, 3, 4, and 5 h were offset for clarity).

were weak because the emission of volatile compounds was not strong at such an early growth stage. The intensities were increased with increasing the growth time because more volatile compounds were produced from the metabolism of the bacteria.

PCA was performed to classify *S.* Enteritidis or *S.* Typhimurium odor from uncontaminated beef odor. Fig. 4 and Fig. 5 show PCA score plots of *S.* Enteritidis odor and *S.* Typhimurium odor classification, respectively. At relatively early growth stages (upto 3 h of incubation), *S.* Enteritidis contamininated beef odors were positioned near the odors of uncontaminated beef in the score plot (Fig. 4). As growth time increases (4-6 h of incubation), *S.* Enteritidis contamininated beef odors were departed away from the odors of uncontaminated beef. PCA score plot shows that the classification *S.* Enteritidis contaminated beef odor was mainly explained by PC1 (56.8%).

S. Typhimurium contamininated beef odor was more distinctive than *S.* Enteritidis contamininated beef odor. *S.* Typhimurium contamininated beef odor could be differentiated from the odor of uncontaminated beef even at relatively early growth stages (Fig. 5). The odor difference between *S.* Typhimurium contamininated and uncontaminated beef was clear after 3 h of incubation. The classification of *S.* Typhimurium contamininated beef odor was also mainly explained by PC1 (73.7%).

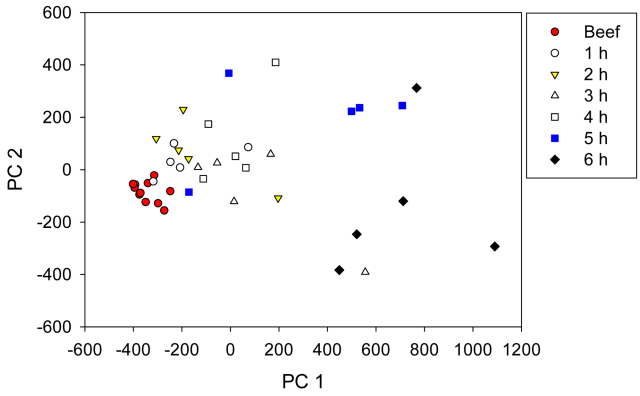

Fig. 4. PCA score plot for the classification of *S.* Enteritidis.

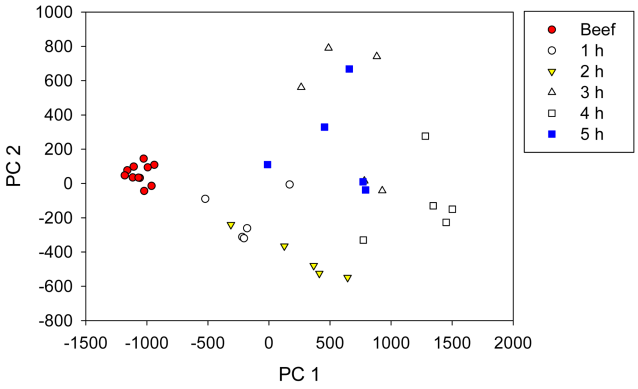

Fig. 5. PCA score plot for the classification of *S.* Typhimurium.

By using PCA, *Salmonella* contaminated beef odor was successfully differentiated from uncontaminated beef odor after 4 h of incubation (Fig. 6). The number of cells enumerated by standard plate count for beef samples incubated 4 hrs after cell inoculation were 2×10^6 CFU/g for both *S.* Enteritidis and *S.* Typhimurium.

A change in odor measured at different incubation times might be explained by activities of the cells. At the initial stage of the cell growth, small amount of chemical is generated by low cell activity. As cell activity increases, more volatile compounds were produced and such a difference between *Salmonella* contaminated beef odor and uncontaminated beef odor in terms of peaks was increased. But the amount of produced chemicals was not proportional to the incubation time. The result was similar to that of other researcher (Tahir and Alocilja, 2003), and the reason is that the volatile compounds were diminishing over time because of deteriorated growth environment

Qualitative analysis of the volatile organic compound isolated from the headspace of *S.* Typhimurium was performed with a mass spectrometer. Compounds identified from the isolates are listed in Table 1. Identified compounds were mostly alcohols. Ethanol (10.2%) was the main compound, followed by 2-propanamine (5.2%), 3-methyl-1-butanol (4.6%), ethanedioic acid (3.2%), 1-decanol (2.7%), ethyl aminopropionate (1.6%), 2-deuterobutane (1.3%), 1-dodecanol (1.2%), 2-methyl-1-butanol (1.0%), 1-octanol (0.6%), and butyl acetate (0.3%). Arnold and Senter (1998) analyzed volatile compounds produced by various species of bacteria with SPME GC-MS. In their research, gas chromatograms of the VOCs isolated from the headspace of *S.* Enteritidis, *Escherichia coli, Listeria monocytogenes, Klebsiella pneumoniae,*

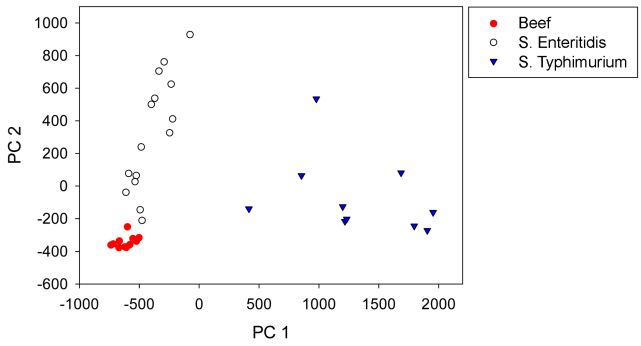

Fig. 6. PCA score plot for the classification of *S.* Enteritidis and *S.* Typhimurium after 4 h of incubation.

Table 1. Volatile organic compounds isolates from the headspace of *S.* Typhimurium cultures by SPME (DVB/CAR/PDMS)

Peak	RT	Area (%)	Compounds
1	2.20	3.2	Ethanedioic acid
2	2.27	10.2	Ethanol
3	2.40	5.2	2-Propanamine
4	2.64	1.6	Ethyl aminopropionate
5	2.76	1.3	2-Deuterobutane
6	5.58	4.6	3-Methyl-1-butanol
7	5.69	1.0	2-Methyl-1-butanol
8	6.08	0.3	Butyl acetate
9	18.80	0.6	1-Octanol
10	26.80	2.7	1-Decanol
11	34.58	1.2	1-Dodecanol

Enterobacter cloacae, Pseudomonas aeruginosa, and *Acinetobacter calcoaceticus*. Results of Arnold and Senter's study shows that dominant compound of the VOCs isolated from the headspace of *S.* Enteritidis, *Escherichia coli, Listeria monocytogenes, Klebsiella pneumoniae, Enterobacter cloacae* was also ethanol. Other compounds isolated from the headspace of *S.* Enteritidis were similar to the compounds isolated from the headspace of *S.* Typhimurium in this research. Those compounds were decanol (3.6%), dodecanol (3.2%), 3-methyo-1-butanol (3.0%), tetradecanol (2.7%), 1-propanol (2.6%), *cis*-7-tetradecene-1-ol (2.2%), octanol (1.6%), and 9-decene-1-ol (0.6%). Other species were distinguished by the production of different compounds such as 3-methyl butanal for *Listeria monocytogenes* and indole for *E. coli*.

The frequent outbreaks of foodborne illness demand rapid detection of foodborne pathogens. Conventional methods for pathogen detection and identification are labor-intensive and take days to complete. Electronic nose technologies have shown great potential for the rapid detection of odor changes related to food safety. A SAW detector-based electronic nose was used to determine safety of beef by analyzing the odors. Detection of *Salmonella* contamination of beef was possible by odor analysis. *Salmonella* contamination of the beef could be classified by PCA after 4 h of incubation. The classification of *Salmonella* contamination was mainly described by the first principal component (PC1). The simplicity and speed of the electronic nose may provide potentials for rapid determination of various foodborne pathogens. Further development of this detection technique will make a significant improvement on food safety programs in the future.

Acknowledgment

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